STUDIES ON VIRUS DISEASES OF FISH

II. Lymphocystis Disease of Fundulus heteroclitus [1]

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At the meeting of the American Society of Zoologists at Indianapolis, December 1937, the author presented a paper and a demonstration concerning "intracellular parasitism in fish producing a gigantic growth of the infected cells." [3] This paper also dealt with parasitic protozoa as well as with the lymphocystis virus disease and the peculiar structure of the lymphocystis cells. With reference to this latter, Dr. Roland Walker, Biological Laboratories, Rensselaer Polytechnic Institute, Troy, New York, sent to the author sections through a tumor of the common salt water killifish *Fundulus heteroclitus*, which he had preserved in August, 1937, at the Marine Biological Laboratory, Woods Hole, supposing that this tumor represented something similar to the demonstrated hypertrophied fish cells.

Studying these preparations I could easily verify that the tumor indeed represents a lymphocystis tumor, never described hitherto on *Fundulus hetroclitus*. I am greatly indebted to Dr. Walker, who decided to leave the description of this interesting observation entirely to me.

The significance of this discovery is not represented so much by the finding of a new host of lymphocystis disease, but by the fact that this fish is known to be easily kept under laboratory conditions. Therefore, it can be hoped that lymphocystis disease could be cultivated in the laboratory for further experimental studies if sufficient attention were paid to a new appearance of this virus disease on *Fundulus heteroclitus*.

The writer estimates from the series of sections that the tumor probably had a diameter of 2 to 3 mm. The localization of the tumor was in the membrane of the tail fin. The preserved fish was the only specimen among 200 *Fundulus heteroclitus* on which Dr. Walker ob-

[1] Studies on virus diseases of fish. I. Lymphocystis disease of the Orange filefish (*Alcutera schoepfii*) appeared in the *Amer. Jour. Hygiene*, Vol. 28, No. 3, 455–462, November, 1938.

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[3] Abstract in *Anat. Rec.*, **70**: No. 1, Suppl. No. 1, 68.

served such a tumor. The tumor, preserved with Bouin's fluid and stained with Harris' hematoxylin, consists of large cells of diameters of 135 to 225 microns, located in the connective tissue of the fin membrane.

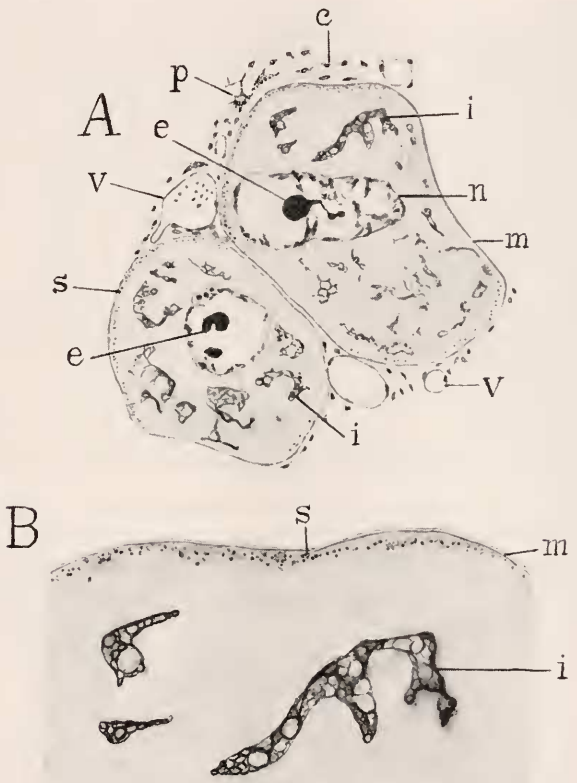

FIG. 1. *A*. Two lymphocystis cells of *Fundulus heteroclitus*. × 210. *B*. Upper part of the upper cell with higher magnification. × 600. *n*, nucleus; *e*, nucleolus; *i*, inclusion bodies; *m*, membrane; *s*, strongly stained granules in the cortex of the lymphocystis cells; *c*, small connective tissue cells; *p*, pigment cell; *v*, vessels.

These large cells (Fig. 1*A*) contain in their cytoplasm the peculiar reticular inclusion bodies which represent the most conspicuous structural element of lymphocystis cells. The inclusions (*i*) appear in the sections as separate coils, but are probably part of a continuous network, similar to those found in the lymphocystis cells of the perches

(*Acerina, Stizostedion*). Figure 1*B* shows with higher magnification their basophilic framework representing a reticular or alveolar structure, strongly stained with hematoxylin. The meshes or alveoles are filled with a paler tingible substance.

These inclusion bodies are so characteristic that the diagnosis of lymphocystis cells is assured without further ado although the second typical structure of the lymphocystis cells, the cell-membrane, shows a remarkably poor development. The cell-membrane appears in Fig. 1*A*

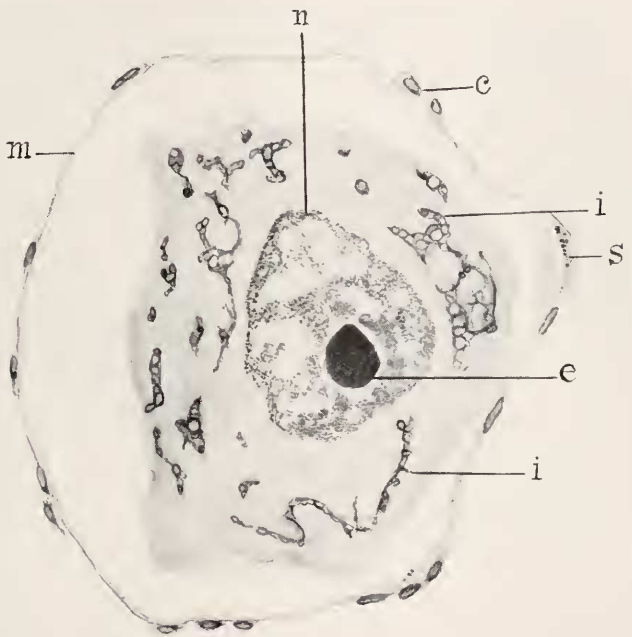

FIG. 2. Lymphocystis cell of *Fundulus heteroclitus*, the cytoplasm of which has withdrawn by shrinking from the membrane (*m*). *n*, nucleus; *e*, nucleolus; *i*, inclusion bodies; *s*, strongly stained granules in the cortex of the lymphocystis cell; *c*, small connective tissue cells. × 600.

only as a contour (*m*) and also with higher magnification (Fig. 1*B*), the configuration of the membrane is not very clear. But cells, in which the cytoplasm has withdrawn from the membrane by shrinking (Fig. 2), show distinctly that the membrane is represented by a cuticula of double contour, evidently homologous to the thicker membranes of the lymphocystis cells of other fish.

The nuclei contain in their peripheral zone a fairly large amount of chromatic substance as bars and fields of granules. One or two nucleoli

(*c*) are to be seen which, as it seems, can give origin to buds (Fig. 1*A*). Just beneath the membrane of the lymphocystis cells a layer of irregular granules (*s*), strongly stained with hematoxylin, could be observed, which, as a rule (Fig. 1*A*), have only developed on those planes of the cells which face the surfaces of the fin membrane. Something similar was seen by the author hitherto only on some degenerating lymphocystis cells of *Pleuronectes flesus,* but there in the whole peripheral zone of the cells.

Because it would be very important for the further research on lymphocystis virus to obtain a stock of lymphocystis-affected fish which might be easily kept in aquaria, the writer attempted to pursue in 1938 the trail found by Dr. Walker. Unfortunately, it seems that the occurrence of lymphocystis disease on *Fundulus heteroclitus* is very rare. Five hundred and fifty specimens of this species, preserved at the Marine Biological Laboratory, Woods Hole, in August 1938, were thoroughly examined without discovering any other case of lymphocystis disease.

Further, there was no success in attempting to transmit to *Fundulus heteroclitus* the lymphocystis disease of the wall-eyed pike perch, *Stizostedion vitreum*, fresh material of which could be procured from the Great Lakes. Corresponding experiments to transmit the *Stizostedion*-disease to the freshwater killifish, *Fundulus diaphanus*, gave the same negative result. The method applied in these experiments consisted in dispersing an emulsion of *Stizostedion*-lymphocystis tumors into the aquarium and in feeding small pieces of the tumors. The *Fundulus*-fish of both species were very eager to swallow the *Stizostedion*-lymphocystis cells, but none of 16 *F. heteroclitus* and of 12 *F. diaphanus* became infected.

Concerning this result, the following facts may be taken into account: (1) The transmission of lymphocystis disease under laboratory conditions was hitherto only successful by keeping diseased and healthy fish together or by the method applied in the *Stizostedion-Fundulus*-experiments which attempted to imitate the natural conditions of infection. (2) This method gave, as a rule, positive results in high percentage in all experiments in which the applied tumor material originated in the same kind of fish to which the treated fish belonged. [See experiments of Weissenberg in Europe on *Acerina cernua* (1914)[4] and *Pleuronectes flesus* (1921)[5] and in this country on *Stizostedion*

[4] Weissenberg, R., 1914. Über infectiöse Zellhypertrophie bei Fischen (Lymphocystiserkrankung). *Sitzungsber. d. Kgl. preuss. Akad. d. Wiss., Physik. mathem. Kl.,* 30: 792–804.

[5] Weissenberg, R., 1921. Lymphocystiskrankheit der Fische. *Handbuch der Pathogenen Protozoen herausgegeben von v. Prowazek-Nöller,* 3: 1344–1380.

vitreum (still unpublished)]. (3) The lymphocystis disease of *Acerina cernua* was discovered by Weissenberg on a Baltic Sea tribe of this species. A freshwater tribe of the same species showed in an infection experiment a lower susceptibility than the original tribe (Weissenberg, 1914, l.c., p. 802). (4) Experiments to infect fishes of other species, genera or families by keeping them together with lymphocystis-affected fish has hitherto always given negative results. But these observations (Joseph, 1918;[6] Weissenberg, 1921, l.c., p. 1348) referred to fish on which lymphocystis disease is not observed in nature.

The negative result in the aforesaid experiments to transmit lymphocystis disease from *Stizostedion vitreum* to *Fundulus heteroclitus* and *diaphanus,* in which experiments a certain susceptibility for the disease could be supposed at least for *F. heteroclitus* on account of the described case of Woods Hole, is in accordance with the interpretation that different kinds of lymphocystis viruses are to be distinguished which are adapted to different fishes.

It would be very desirable to pay attention to further cases of lymphocystis disease of *Fundulus heteroclitus* and to keep affected specimens together with healthy fish of this species for cultivating a stock of lymphocystis-affected fish, which could easily be kept under laboratory conditions and therefore would be well adapted for the further study of this interesting virus disease in this country.

Summary

The first and until now solitary case of lymphocystis disease in *Fundulus heteroclitus* is described. *Fundulus heteroclitus* and *F. diaphanus* proved not susceptible to the virus of the lymphocystis disease of *Stizostedion vitreum* in infection experiments.

[6] Joseph, H., 1918. Untersuchungen über Lymphocystis Woodc. *Arch. f. Protist.,* Bd. 38.